COMPOSITION, METHOD, AND KIT FOR ALPHA-1 PROTEINASE INHIBITOR

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Application No. 61/257,711 filed Nov. 3, 2009~~, which is herein incorporated by reference in its entirety~~.

FIELD OF THE INVENTION

The present invention relates to compositions comprising alpha 1-proteinase inhibitor (API), in particular stable API compositions. The present invention also relates to methods and kits for providing API to a subject, in particular for providing to the subject a therapeutically or prophylactically effective amount of API.

BACKGROUND OF THE INVENTION

API deficiency is a relatively common genetic disorder that predisposes affected individuals to liver disease and/or pulmonary emphysema. The most common type of API deficiency termed protease inhibitor type Z (PiZ), is transmitted as an autosomal recessive trait and affects approximately 1 in 1700 live births in most Northern European and North American populations. The PiZ mutation is a single nucleotide substitution that results in a single amino acid substitution (glutamate 342 to lysine).

Human API has 394 amino acids, one cysteine residue, and three carbohydrate side chains, giving an overall molecular weight of 52 kDa. A single reactive center loop (RCL) is located at a Met-Ser sequence at position 358-359. The tertiary structure of API contains 8 well-defined α-helices (A-H) and 3 large β-sheets (A-C). It is believed that serpins function via irreversible "suicide-substrate" mechanism. Upon cleavage by a protease, the serpin undergoes a conformational change, whereby the RCL is cleaved and inserted into the center of the A β-sheet, and the protease is translocated to the distal end of the molecule. This structural transition results in stabilization of the serpin and extensive distortion of the protease's tertiary structures, which inactivates its catalytic machinery. It is believed that the biological activity of API can be effected by, *e.g.*,

continuous liquid feed. Using these methods, the composition comprising API can be dried into fine particles, preferably with suitable types and amounts of excipients such as that can act as water-replacing agents.

Pressurized metered dose inhalers are now well known in the art. Any pressurized metered dose inhaler that is suitable for application of drugs to the lungs or nose of a patient can be used. The aerosol formulation and metering valve can be selected to provide a therapeutically or prophylactically effective amount of the API.

A. API

The term "API," as used herein, is intended to be broad unless specifically stated otherwise. The term refers to all naturally occurring polymorphs of API. The term also includes functional fragments of API, chimeric proteins comprising API or functional fragments thereof, homologs obtained by analogous substitution of one or more amino acids of API, and species homologs. The term also refers to all API polypeptides that are a product of recombinant DNA technology including an API that is a product of transgenic technology. For example, the gene coding for API can be inserted into a mammalian gene encoding a milk whey protein in such a way that the DNA sequence is expressed in the mammary gland as described in, *e.g.*, U.S. Patent No. 5,322,775, ~~which is herein incorporated by reference for its~~ teaching ~~of~~ a method of producing a proteinaceous compound. The term also refers to all API proteins synthesized chemically by methods known in the art such as, *e.g.,* solid-phase peptide synthesis. The term also refers to API prepared from plasma. The term also refers to API that can be obtained commercially. The API can correspond to a human or a non-human API.

In one embodiment, the API is plasma-derived API. In another embodiment, the API is prepared from Cohn Fraction IV-1 paste. In other embodiments, the API is prepared from an albumin-depleted plasma fraction, a Cohn V precipitate, or a pre-purified API preparation fraction. U.S. Patent No. 6,974,792 ~~is herein incorporated by reference for its~~ teach<u>es</u>~~ing of~~ a method of preparing API.

In other embodiments, the API is recombinant API. Amino acid and nucleotide sequences for API and/or production of recombinant API are described by, *e.g.,* U.S. Patent Nos. 4,711,848; 4,732,973; 4,931,373; 5,079,336; 5,134,119; 5,218,091; 6,072,029; and Wright *et al.*, Biotechnology, 9:830 (1991); and Archibald *et al.*, PNAS,

87:5178 (1990), each of which ~~is herein incorporated by reference for its~~ teaches~~ing~~ of API sequences, recombinant API, and/or recombinant expression of API.

In one embodiment, the composition is characterized as comprising an API having a greater than 90% purity. In other embodiments, the API has a purity greater than 95%, preferably at least about 99%. In some embodiments, at least about 50%, illustratively, about 50% to about 100%, about 60% to about 90%, about 70% to about 80% of all API in the composition is active API.

In one embodiment, the composition comprises a therapeutically effective amount of API. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as, for example, reduction or inhibition of emphysema associated with congenital deficiency of API. A therapeutically effective amount of API can vary according to factors such as the disease state, age, sex, and weight of the individual subject, and the ability of the API to elicit a desired response in the subject. A therapeutically effective amount also can be one in which any toxic or detrimental effects of API are outweighed by the therapeutically beneficial effects.

In other embodiments, the composition comprises a prophylactically effective amount of API. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as, for example, preventing or inhibiting emphysema associated with congenital deficiency of API. A prophylactically effective amount can be determined as described above for the therapeutically effective amount.

One factor that may be considered when determining a therapeutically or prophylactically effective amount of API is the concentration of functionally active API expressed in a biological compartment of the subject receiving the treatment, such as, for example, in the lower respiratory tract or the epithelial lining fluid (ELF) of the subject. Another factor that also may be considered when determining a therapeutically or prophylactically effective amount of API is the pharmacology (*e.g.*, pharmacokinetics) of the API.

The pharmacokinetics of API refers to the concentration or levels of API in the subject's blood. Pharmacokinetic parameters or measures of API levels in the blood

characterized as exhibiting a hydrophobicity of less than zero according to a consensus hydrophobicity scale.

In one embodiment, the at least one amino acid is present in the composition in a total amino acid amount that is sufficient to provide a stable API composition, for example as determined relative to an API composition not having the at least one amino acid.

In another embodiment, the total amino acid amount is an amount sufficient such that the API retains at least about 5% of an API activity for a period of time at a temperature, illustratively, at least about 5% to about 100%, about 10% to about 95%, about 20% to about 90%, about 30% to about 80%, about 40% to about 70%, and about 50% to about 60% of an API activity for a period of time at a temperature. In some embodiments, the period of time and/or the temperature is characterized as a storage time and/or temperature (*e.g.,* shelf-life). In one embodiment, the period of time is at least about: 1 day, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 8 months, 1 year, 2 years, and 3 years. In another embodiment, the temperature is equal to or greater than about: -120 °C, -70 °C, -20 °C, 0 °C, 5 °C, 25 °C, 37 °C, 40 °C, and 50 °C.

In one embodiment, the API activity can be determined by inhibition of porcine pancreatic elastase as described by, *e.g.,* Coan *et al.*, Vox Sang., 48:333 (1985)~~, which is herein incorporated by reference in its entirety~~. In some embodiments, the API activity can be assessed as potency or as toxicity by an *in vitro*, *in vivo,* and/or *ex vivo* determination (*e.g.* therapeutic index, LD_{50}, ED_{50}, etc.) in an animal. For example, following serial dilutions from a bulk or stock solution, or reconstitution of a lyophilized, or vacuum dried API containing composition, the API present in the reconstituted or aqueous composition has (in the presence of the at least one amino acid and/or the total at least one amino acid amount) greater than at least about 5% to about 100% of the potency or toxicity that the biologically active API had prior to being incorporated into the composition.

In other embodiments, stability of the API can be determined by, for example, determining degradation of the API including, but not limited to, formation of oxidation, proteolytic, and/or deamidation products, changes in molecular weight distribution,

We claim:

1. A composition comprising:

(a) an alpha 1-proteinase inhibitor (API); and

(b) at least one amino acid, wherein the at least one amino acid is present in the composition in a total amino acid amount of ~~about~~ 0.01 M to ~~about~~ 3 M such that the API retains at least 50% of its activity when the composition is exposed to a temperature for a period of at least 6 months,

wherein the composition does not contain a sugar or contains a sugar in a total amount of less than 20% w/v.

2. The composition of claim 1, wherein the temperature is ~~about~~ 5 °C to ~~about~~ 40 °C.

3. The composition of claim1, wherein the at least one amino acid is a neutral or a hydrophilic amino acid.

4. The composition of claim 1, wherein the at least one amino acid is selected from the group consisting of: alanine, threonine, serine, hydroxyproline, glycine, proline, leucine, and histidine.

5. The composition of claim 1, wherein the amino acid is alanine.

6. The composition of claim 1 further comprising one or more excipients.

7. The composition of Claim 6~~-1~~, wherein the one or more excipients is selected from the group consisting of: sucrose, mannitol, glycerol, sorbitol, dextran, trehalose, hydroxyethyl starch (HES), and 1,2-propanediol.

8. The composition of claim 1, wherein the composition further comprises a pharmaceutically acceptable carrier.

9. The composition of claim 1, wherein the composition is suitable for intravenous administration to a subject.

10. The composition of claim 1, wherein the at least one amino acid is present in the composition in a total amino acid amount sufficient to maintain total protein aggregation at less than ~~about~~ 5% following exposure of the composition to a temperature for a period of time.

11. The composition of claim 10, wherein the temperature is ~~about~~ 25 °C, wherein the period of time is ~~about~~ 6 months.

12. The composition of claim 10, wherein the temperature is ~~about~~ 5 °C, wherein the period of time is ~~about~~ 6 months.

13. The composition of claim 12, wherein the at least one amino acid is present in the composition in a total amino acid amount sufficient to maintain total protein aggregation at less than ~~about~~ 2% following exposure of the composition to the temperature for the period of time.

14. The composition of claim 10, wherein the pH of the composition is ~~about~~ 6.3 to ~~about~~ 7.4.

15. The composition of claim 10, wherein the pH of the composition is ~~about~~ 6.7 to ~~about~~ 7.1.

16. The composition of claim 1, wherein the composition is in lyophilized form.

17. A kit comprising the composition of claim 1 as a pharmaceutical composition suitable for intravenous administration.